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(54) Title: NUCLEIC ACID SEQUENCES CODING FOR OR COMPLEMENTARY TO NUCLEIC ACID SEQUENCES CODING FOR INTERLEUKIN 9 RECEPTOR

[illegible]

(57) Abstract

The invention describes nucleic acid sequences which code for the interleukin 9 receptor (IL9-R) molecule. These sequences may be used as probes to identify cells expressing the molecule, and as agents to transfect recipient cells.

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NUCLEIC ACID SEQUENCES CODING FOR OR COMPLEMENTARY TO
NUCLEIC ACID SEQUENCES CODING FOR INTERLEUKIN 9 RECEPTOR
FIELD OF THE INVENTION

This invention relates to the reception of the cytokine known as interleukin 9 by cells, via its receptor. More particularly, it relates to the isolation of nucleic acid sequences which code for interleukin 9 receptor molecules ("IL-9R" hereafter). These sequences can be used, e.g. as a source for IL-9 receptor, and as probes for cells which respond to the cytokine. The complementary sequences can be used to inhibit expression as well as to probe for the coding sequences.

BACKGROUND AND PRIOR ART

The last decade has seen knowledge of the immune system and its regulation expand tremendously. One area of particular interest has been that of research on the proteins and glycoproteins which regulate the immune system. Perhaps the best known of these molecules, which are generically referred to as "growth factors", "cytokines", "leukotritins", "lymphokines", etc., is interleukin-2 ("IL-2"). See, e.g., U.S. Patent No. 4,778,879 to Mertelsmann et al.; U.S. Patent No. 4,490,289, to Stern; U.S. Patent No. 4,518,584, to Mark et al.; and U.S. Patent No. 4,851,512 to Miyaji et al. Additional patents have issued which relate to interleukin 1 - ("IL-1"), such as U.S. Patent No. 4,808,611, to Cosman. The disclosure of all of these patents are incorporated by reference herein.

In order for molecules such as IL-2 and IL-1 to exert their effect on cells, it is now pretty much accepted that these must interact with molecules, located on cell membranes, referred to as receptors. Patents which exemplify disclosures of interleukin receptors include Honjo et al., U.S. Patent No. 4,816,565; and Urdal et al., U.S. Patent No. 4,578,335, the disclosures of which are incorporated by reference. Recently, Fanslow, et al., Science 248: 739-41 (May 11, 1990) presented data showing that the effect of IL-1 in vivo could be regulated via the

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administration of a soluble form of its receptor. The last paragraph of the Fanslow paper, the disclosure of which is incorporated by reference, describes the types of therapeutic efficacy administration of soluble IL-1 receptor ("IL-1R") is expected to have.

The lymphokine IL-9, previously referred to as "P40", is a T-cell derived molecule which was originally identified as a factor which sustained permanent antigen independent growth of T4 cell lines. See, e.g., Uyttenhove, et al., Proc. Natl. Acad. Sci. 85: 6934 (1988), and Van Snick et al., J. Exp. Med. 169: 363 (1989), the disclosures of which are incorporated by reference, as is that of Simpson et al., Eur. J. Biochem. 183: 715 (1989).

The activity of IL-9 was at first observed to act on restricted T4 cell lines, failing to show activity on CTLs or freshly isolated T cells. See, e.g., Uyttenhove et al., supra, and Schmitt et al., Eur. J. Immunol. 19: 2167 (1989). This range of activity was expanded when experiments showed that IL-9 and the molecule referred to as T cell growth Factor III ("TCGF III") are identical. IL-9 enhances the proliferative effect of bone marrow derived mast cells to "IL-3", as is described by Hültner et al., Eur. J. Immunol. and in U.S. patent application Serial Number 498,182 filed March 23, 1990 the disclosures of both being incorporated by reference herein. It was also found that the human form of IL-9 stimulates proliferation of megakaryoblastic leukemia. See Yang et al., Blood 74: 1880 (1989). Recent work on IL9 has shown that it also supports erythroid colony formation (Donahue et al., Blood 75(12): 2271-2275 (6-15-90)); promotes the proliferation of myeloid erythroid burst formation (Williams et al., Blood 76: 306-311 (9-1-90)); and supports clonal maturation of BFU.E's of adult and fetal origin (Holbrook et al., Blood 77(10): 2129-2134 (5/15/91)). Expression of IL9 has also been implicated in Hodgkin's disease and large cell anaplastic lymphoma (Merz et al., Blood 78(8): 1311-1317 (9-1-90)).

The art teaches the cloning of receptors for various

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members of the interleukin family. Moseley et al. Cell 59: 335-348 (1989), teach the isolation of cDNA coding for IL-4 receptors, and analysis of both genomic DNA and RNA for these molecules. To do this, Moseley et al. worked with cells exhibiting up to 1 million receptor molecules per cell, and an N-terminal amino acid sequence for IL-4 receptor. Holmes et al., Science 253: 1278-1280 (1991), and Murphy et al., Science 253: 1280-1282 (1991) discuss cDNA for the IL-8 receptor. Murphy et al. proceeded via hybridization studies, using an oligonucleotide probe based upon rabbit IL-8R amino acid sequences to isolate the human counterpart. Holmes et al. used human neutrophil cDNA libraries followed by transfection in COS cells.

Gillis, "T-cell Derived Lymphokines" in Paul, ed., Fundamental Immunology, Second Edition (New York, 1989), at pages 632 et seq. gives an overview of interleukin receptors. This reference describes cDNA for the IL1 receptor, the IL2 receptor and the IL-6 receptor.

These studies indicate that several factors are important in attempting to identify and isolate a nucleic acid sequence coding for an interleukin receptor. Ideally, one has both the amino acid sequence for the receptor and a cell type with a high degree of expression of the receptor molecule.

In the case of the interleukin 9 receptor, while Druez et al., J. Immunol. 145: 2494-2499 (1990) have identified and characterized the receptor, an amino acid sequence of the molecule is not yet available. In addition, very few cell types are known which express IL9-R (Druez, supra), and those that do, express it at very low levels. Thus, it is surprising that it is now possible to identify and to isolate nucleic acid sequences which code for the interleukin 9 receptor. This is the key feature of the invention described herein, as will be seen from the disclosure which follows.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 presents Scatchard analysis of expression of

murine IL9 receptor following transfection of COS cells.

Figure 2 aligns deduced human and murine IL-9R amino acid sequences.

Figure 3 compares the response of TS1 cells, both before and after transfection with DNA coding for human IL-9R.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Example 1

The murine T cell clone, TS1, described by, e.g., Uyttenhove et al., Proc. Natl. Acad. Sci. 85: 6934-6938 (1988) the disclosure of which is incorporated by reference, expresses approximately 200 high affinity binding sites for IL-9, i.e., it expresses the IL-9 receptor molecule. See Druez et al., J. Immunol. 145: 2494-2499 (1990). This cell line, while presenting few receptor molecules does show the highest density of IL9R of all cells tested, and thus was selected as a source of mRNA for constructing a cDNA library.

Poly(A)+ mRNA was extracted from TS1 cells, and was then converted to double stranded cDNA using random hexanucleotide primers, following Grubler et al, Gene 25: 263-269 (1983), the disclosure of which is incorporated by reference.

Following this, EcoRI adaptors were attached, and any cDNA larger than 1.5 kilobases was isolated by fractionation on a 5-20% potassium acetate gradient, following Aruffo et al., Proc. Natl. Acad. Sci. 84: 8573-8577 (1987).

The size selected cDNA was then inserted into the ECORI site of expression vector pCDSR α taught by Takebe et al., Mol. Cell Biol. 8: 466-472 (1988). This was then transfected into E. coli strain XL1-blue using standard transformation procedures. (Maniatis). In order to screen for clones expressing IL-9R, plasmid DNA from the cDNA library was tested for the ability to express IL-9 binding activity by expression in COS cells. Basically, the cDNA library was subfractionated into 100 pools of about 500 clones each, and the DNA was transfected using the DEAE-dextran-chloroquine method of Aruffo et al., supra, into 1.5

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$\times 10^5$ COS cells, seeded on glass microscope slides. Cells were allowed to grow for 2-3 days, and were then tested for expression of IL-9R with ^{125}I labelled, purified recombinant murine IL9. This labeled material was prepared following Bolton et al., Biochem. J. 133: 529-539 (1973). The cells were incubated for three hours at 20°C with 0.2 nM of this material, washed briefly, fixed, and then dipped into liquid photographic emulsion. The slides were exposed for 10 days, then developed and examined microscopically for autoradiographic grains.

This screening resulted in two positive pools out of 100. One positive pool showed a single positive cell, and the second one showed 33 positive cells. This latter pool was selected for further testing, and was divided, first into 100 pools of 15 clones each, after which a single positive pool was selected, and divided into 100 single clones.

Example 2

Following the separating and replating described at the end of example 1, supra, the screening methodology described therein was employed on the replated cells, and led to identification of a clone containing a plasmid referred to as p9RA1. Since the "source" plasmid pCDSR α was known and characterized, it was possible using standard methodologies to identify the insert as 1900 base pairs in length.

Example 3

Using the p9RA1 1900 base pair segment as a probe, additional screening was carried out to identify additional murine IL9R receptor cDNA clones. The methodology followed was that of Maniatis et al., Molecular Cloning, a Laboratory Manual (Cold Spring Harbor Laboratory, New York, 1982), where the p9RA1 probe was hybridized to two further cDNA libraries which were generated in the BstXI site of vector pCDM8 (Aruffo et al, supra), using oligo T or random primers, followed by high stringency washes.

This methodology resulted in the identification of six additional clones. Two of these were oligo-dT primed cDNAs, and are referred to as p9RB1, and p9RB3, and four random

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primed clones p9RC2, p9RC3, p9RC4 and p9RC9. The sizes of these clones are as follows:

p9RB1	1600 bp
p9RB3	900 bp
p9RC2	2000 bp
p9RC3	1000 bp
p9RC4	3000 bp
p9RC9	2100 bp.

Example 4

In order to make sure that clone p9RA1 and all subsequent clones did in fact express IL9R, Scatchard analysis was carried out on transfected COS cells, following Goodwin et al., Cell 60: 941-951 (1990). This analysis, shown in figure 1, identified a single class of binding sites with a K_d of 194 pM, when p9RA1 was used. This is slightly higher than the dissociation constant measured on TS1 cells previously, i.e., 67 pM.

When the largest cDNA was tested (i.e., the C4 clone), high affinity binding sites for IL9 were also identified, with a K_d of 126 pM.

Example 5

Following the isolation of murine clones, tests were also carried out to isolate analogous human material. To do this, a megakaryoblast cell line, i.e., Mo7E was used as a source of mRNA to make double stranded cDNA as per example 1. The plasmid pRC/RSV was used to receive the cDNA. This cDNA library was screened, using p9RA1 as a probe, and hybridization was carried out using the same conditions described supra, except washes were carried out at low stringency (2 x SSC, 0.1% SDS, 55°C). Six clones were isolated, i.e., ph9RA2, 3, 4, 5, 6 and 9, and sequenced. The clone ph9RA3 contained a 1566 base pair open reading frame, which showed 66% identity with murine p9RC4. The deduced murine and human protein sequences are shown in figure 2, with a 53% identity over 522 amino acids.

Example 6

In order to test whether clone ph9RA3 actually did code

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for a human IL9 receptor, the clone was transfected into murine cell line TS1, using double pulse electroporation. In brief, 5×10^6 TS1 cells were resuspended at 37°C in 0.8 ml of Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, 50 mM 2-mercaptoethanol, 0.55 mM L-arginine, 0.24 mM L-asparagine, and 1.25 mM L-glutamine. Plasmid DNA (50 ug) was added to the cells in 0.4 cm cuvettes just before electroporation. After a double electric pulse (750 V, 7452Ω, 40 μF and 100 V, 74Ω, 2100 μF), cells were immediately diluted in fresh medium supplemented with murine IL9. After 24 hours, cells were washed and cultured in the presence of G418, and mouse IL9. These conditions resulted in a frequency of transfection of approximately 1/10,000. Following selection with G418, transfected cells were maintained in human IL9, and a TS1 proliferation assay was performed using the methodology of Uyttenhove et al., Proc. Natl., Acad. Sci. USA 85: 6934-6938 (1988). If the cDNA expresses hIL9R, then cells should proliferate, while those which do not contain it should not.

Figure 3 shows that original TS1 cells, unresponsive to 100 units/ml of human IL9, became responsive and proliferated after transfection with the human IL9R cDNA.

Example 7

The sequence of clone p9RC4, presented as SEQ ID NO: 1, shows an open reading frame coding a 468 amino acid protein. The deduced amino acid sequence predicts two hydrophobic regions, one of which spans amino acids 15-40, and probably represents a signal peptide. The probability weight matrix of von Heyne, Nucl. Acids Res. 14: 4683-4690 (1986) predicts a cleavage site for the signal peptide between positions 37 and 39. The second hydrophobic domain spans amino acids 271-291. This is presumed to constitute the transmembrane domain.

The putative extracellular domain contains 233 amino acids, including 6 cysteine residues and two potential N-linked glycosylation sites at positions 116 and 155. A 'WSEWS' motif, i.e., "Trp-Ser-Glu-Trp-Ser", typical of the

hematopoietin receptor superfamily described by Idzerda et al., J. Exp. Med. 171: 861-873 (1990), is found at positions 244-248.

The cytoplasmic portion of the molecule is characterized by a high percentage of serine (13%), and proline (12.4%), as well as three potential protein kinase C phosphorylation sites at positions 294, 416 and 465.

Comparison of the various clones indicates that p9RA1 and p9RB3 contain an additional glutamine between position 192 and 193 as compared to p9RC4, but without a frameshift. This residue lies in the extracellular domain, but as example 4, supra shows, it does not appear to affect the affinity for ligand. There is a 22 nucleotide deletion at this position in p9RC2. These features, and a potential intronic sequence in p9RC9, suggest alternate splicing events.

The analysis of p9RB3 implies the existence of a soluble form of IL9R. The cDNA for this clone contains a large part of extracellular domain, but lacks nucleotides 651-1719, which code the end of the N-terminal domain, the transmembrane and the cytoplasmic domain.

Clone p9RA1 is different from all other clones in that there is a stop codon after alanine (378), which is followed by a 736 base pair sequence unrelated to any other cDNA's sequenced.

The sequences for the murine cDNA described in this example is provided as follows:

p9RC4 (SEQ ID NO: 1)
p9RA1 (SEQ ID NO: 2)
p9RB3 (SEQ ID NO: 3).

Example 8

The cDNA for human IL9-R was also analyzed. As indicated supra, clone ph9RA3 showed 66% identity with murine p9RC4 and 53% homology on the amino acid sequence level. A putative cleavage site is positioned between amino acids 39 and 40. This site is conserved between species, as is the transmembrane domain, the two potential N-glycosylation

sites, and the consensus sequence for the hematopoietic superfamily, all of which are described in Example 7.

The cytoplasmic portion of the protein seemed less conserved, and was much larger (231 amino acids) than the murine counterpart (177 residues). Due to a stretch of 9 consecutive serines in positions 431-439, there is a high percentage of serine in the molecule (11.2%).

Clones ph9RA2, 4, 6 and 9 confirmed the sequence derived from ph9RA3. The clone ph9RA5, however, has an 85 nucleotide deletion in positions 1063-1147, suggesting a truncated protein. The putative truncated protein would be 307 amino acids long, and contain the complete extracellular and transmembrane regions of IL9-R, 5 amino acids of the cytoplasmic domain, and 11 unrelated residues.

The clone referred to as pH9RA6 contains a short intervening sequence at the beginning of the DNA, which leads into a stop codon, in frame with the normal initiative codon. It also creates a new ATG triplet in frame with the downstream portion of the coding sequence. In the IL9R molecule, this yields a transcript with a unique N-terminal sequence, the rest of the sequence being identical to pH9RA3. Comparison of pH9RA6 and pH9RA3 shows that, after the initial methionine common to both clones, pH9RA6 contains an insert of 22 amino acids. These are followed by the sequence "GWTLESE ..." which is the sequence beginning at position 10 of pH9RA3.

The nucleic acid sequences for ph9RA3, ph9RA5 and pH9RA6 are presented as SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

The foregoing teaches the isolation of a nucleic acid sequence which codes for the interleukin-9 receptor. Both murine and the homology found therebetween (53%, with up to 67% in the extracellular region) suggests that nucleic acid sequences coding for IL9-R from other species could also be identified.

The following data deal with cDNA, but it will be seen that the sequences of the cDNA put one in position of mRNA,

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as the latter can be derived from the former based on well known rules regarding construction of the sequences. Given the cDNA information, it is presumed that one could also secure the genomic analog of the cDNAs.

The information provided herein also teaches construction of vectors, such as plasmids, which contain the nucleic acid sequences of interest, i.e., those coding for mammalian IL9R. Such vectors may contain components in addition to the coding sequence, such as promoters operably linked to the coding sequence, "markers", such as genes for antibiotic resistance or selection, including the thymidine kinase or "TK" gene, as well as others which will be known to the skilled artisan. The nucleic acid sequences and vectors may be used - as has been shown - to transfect various cell types, such as "COS", "CHO", Spodoptera frugiperda or other insect cell lines. The sequences, either alone or in appropriate vectors, can be used to transfect a panoply of prokaryotic and eukaryotic cells.

The isolation of nucleic acid sequences coding for the IL9 receptor makes it possible for investigators to carry out several lines of investigation which were not possible or much more difficult without these. For example, as pointed out supra, even on these cells which express it best, expression of IL-9R is low. Isolation of the gene makes it possible to transfect recipient cells, followed by overexpression, amplification, etc. This leads to sufficient expression on cell surfaces to permit immunization with these cells, and generation of an immunogenic response to IL-9R, including the production of antibodies. Isolation of the antibody producing cells, followed by standard techniques of hybridoma biology leads to production of IL-9R specific monoclonal antibodies.

The antibodies produced, be they polyclonal or monoclonal, can then be used in therapeutic methods to block IL-9 from binding to IL-9R molecules. As binding of IL-9 to cell surfaces is implicated in several pathological conditions, this is an important therapeutic goal.

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In addition IL-9R specific antibodies can be used for both qualitative and quantitative measurement of IL-9R expression on cells, following known immunoassay protocols.

The examples supra show the existence of a soluble form of IL-9R. As with other soluble interleukin receptor molecules (see Fanslow et al., supra), this molecule can be used to prevent the binding of IL-9 to cell bound receptor, and thus interfere with the affect of IL-9 on a cell type, subpopulation, etc. As such, soluble IL-9R may be said to be an antagonist for IL-9.

Recent work has shown that the soluble form of one interleukin receptor, i.e., IL-6R, functions as an agonist. See Taga et al., Cell 58: 573-591 (8-11-89). The soluble form of IL-9R might function in a similar manner. In addition the IL-9R molecule, either the soluble form or a solubilized form of the molecule may be used as an immunogen for generation of IL-9R specific antibodies. Either the entire receptor molecule, or an immunogenic portion thereof, can be used in an appropriate animal, such as a mouse, rabbit or guinea pig, to generate an immune response which includes antibody formation. The antibodies can then be purified using standard techniques. Alternatively, antibody producing B cells can be isolated and utilized in any of the standard methods for producing hybridomas, so as to lead to the generation of IL-9R specific monoclonal antibodies.

An assay is described supra, in Example 6, in which IL-9R cDNA expression is assayed by measuring the responsiveness of a transfected cell line to IL9. This assay methodology provides a means for screening for various agonists and antagonists. In brief, a transfected cell sample containing a sequence coding for IL9R is contacted with a compound of interest. If the compound is an agonist, it will bind to the IL-9R molecule on the cell surface, and lead to the series of events usually associated with IL-9/IL-9R binding. To the same end, an antagonist can be assayed by combining the compound of interest with IL-9 and the cell sample to determine whether the IL-9 has diminished

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impact, or no impact. The assay for agonist/antagonist may be viewed as part of a broader invention wherein one may assay for molecules which compete for binding to IL-9R.

In addition to the coding sequences discussed herein, the invention also embraces sequences complementary to the coding sequences. These complements, which can be derived from the coding sequences themselves, may be used, e.g., as probes or as "anti-sense" inhibitors to prevent expression of the IL9R coding sequences. Other aspects of the invention will be clear to the skilled artisan, and do not require elaboration here.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

SEQ ID NO: 1

CTCC

ATG GCC CTG GGA AGA TGC ATT GCG GAA GGT TGG ACC TTG GAG
AGA GTG GCG GTG AAA CAG GTC TCC TGG TTC CTG ATC TAC AGC
TGG GTC TGC TCT GGA GTC TGC CGG GGA GTC TCG GTC CCA GAG
CAA GGA GGA GGA GGG CAG AAG GCT GGA GCA TTC ACC TGT CTC
AGC AAC AGT ATT TAC AGG ATC GAC TGC CAC TGG TCG GCT CCA
GAG CTG GGC CAG GAA TCC AGG GCC TGG CTC CTC TTT ACC AGT
AAC CAG GTG ACT GAA ATC AAA CAC AAA TGC ACC TTC TGG GAC
AGT ATG TGT ACC CTG GTG CTG CCT AAA GAG GAG GTG TTC TTA
CCT TTT GAC AAC TTC ACC ATC ACA CTT CAC CGC TGC ATC ATG
GGA CAG GAA CAG GTC AGC CTG GTG GAC TCA CAG TAC CTG CCC
AGG AGA CAC ATC AAG TTG GAC CCA CCC TCT GAT CTG CAG AGC
AAT GTC AGC TCT GGG CGT TGT GTC CTG ACC TGG GGT ATC AAT
CTT GCC CTG GAG CCA TTG ATC ACA TCC CTC AGC TAC GAG CTG
GCC TTC AAG AGG CAG GAA GAG GCC TGG GAG GCC CGG CAC AAG
GAC CGT ATC GTT GGA GTG ACC TGG CTC ATC CTT GAA GCC GTC
GAA CTG AAT CCT GGT TCC ATC TAC GAG GCC AGG CTG CGT GTC
CAG ATG ACT TTG GAG AGT TAT GAG GAC AAG ACA GAG GGG GAA
TAT TAT AAG AGC CAT TGG AGT GAG TGG AGC CAG CCC GTG TCC
TTT CCT TCT CCC CAG AGG AGA CAG GGC CTC CTG GTC CCA CGC
TGG CAA TGG TCA GCC AGC ATC CTT GTA GTT GTG CCC ATC TTT
CTT CTG CTG ACT GGC TTT GTC CAC CTT CTG TTC AAG CTG TCA
CCC AGG CTG AAG AGA ATC TTT TAC CAG AAC ATT CCA TCT CCC
GAG GCG TTC TTC CAT CCT CTC TAC AGT GTG TAC CAT GGG GAC
TTC CAG AGT TGG ACA GGG GCC CGC AGA GCC GGA CCA CAA GCA
AGA CAG AAT GGT GTC AGT ACT TCA TCA GCA GGC TCA GAG TCC
AGC ATC TGG GAG GCC GTC GCC ACA CTC ACC TAT AGC CCG GCA
TGC CCT GTG CAG TTT GCC TGC CTG AAG TGG GAG GCC ACA GCC
CCG GGC TTC CCA GGG CTC CCA GGC TCA GAG CAT GTG CTG CCG
GCA GGG TGT CTG GAG TTG GAA GGA CAG CCA TCT GCC TAC CTG
CCC CAG GAG GAC TGG GCC CCA CTG GGC TCT GCC AGG CCC CCT
CCT CCA GAC TCA GAC AGC GGC AGC AGC GAC TAT TGC ATG TTG
GAC TGC TGT GAG GAA TGC CAC CTC TCA GCC TTC CCA GGA CAC
ACC GAG AGT CCT GAG CTC ACG CTA GCT CAG CCT GTG GCC CTT
CCT GTG TCC AGC AGG GCC TGA
CACCTACCAA GGGATGTGGG CATTCTCTTC CCTCCTATCC TCGGATGGCA
CCAGACACAG TCTCTGCGTG TCTCTGCTAG GTGCACCATG TCTGTTTTGG
GGAGATGAAC GAAAGGCCCC AGGCTGACCC TGGGGTGCCT GTGGAACCTCC
GGAGAGGAGG CAGCTGTGCA CGGATCAGAG GCAATGCGGA TGGAAAGCAGT
AGACTGTGCC TTACCCCCCT GCTCTGCCTT TGTGGTGGGG ATGCCTCCAG
GGTCAGCATC TTAACATCGC CTTGCTTCTT CTTGTCTTTC TGGCTCTGTC
CCAGGCCTGA AAAAAGAATG TGACAAGCAG CCTGGTCTGT TCTTCCACCC
CTAAAGGGCT GGCCTGGGCC CAGGGACACT GATGAGACAA CATTGGTGAA
GTGTCCCTTT TCAGTGCCTT TCCCATTAAAG ACCAGAAGGG ACGCTTTTGA
CTGCAGGCTG TGGGTGGCTG GGTACGGAGG GAATGATGGA GCTTTGAGCA
GGTGGGGTTG TCCATCTTTG AGCTTTTGGG GTTCCAAGAT CAGCTGGAAG
GAGTCTCACC GACTGATTCA AAGAAGTCTT ACCCATCTGT GATATTTTCT
TTCCTGGTGC CGTGATAAAA CACCGTGACC AAAAATGACT TACAAAAGGA
AGAGTTGGCT TGGTTTAAGG TTCCAGAGGT GTGGAGACAT GGCAGCCAGC
GGCACACATG GCAGTGAGGA CAGGAAGCTG AGAGCTCACA TCTCAACCAA
AAGTTGAGTG AACTGAAAGT ACTATCCCCC CCCCCACCCC AACTCCAGCA
AGGCTCCACC CCCCTGAAGG TTCCATGCCT CCCTAAACAG CTCGGCCAAA
TAGAGACCAA GTGTTCAAAT

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SEQ ID NO: 2

CACCTCCTGG CTGGGGCTGC CTGAGACTCT CC
ATG GCC CTG GGA AGA TGC ATT GCG GAA GGT TGG ACC TTG GAG
AGA GTG GCG GTG AAA CAG GTC TCC TGG TTC CTG ATC TAC AGC
TGG GTC TGC TCT GGA GTC TGC CGG GGA GTC TCG GTC CCA GAG
CAA GGA GGA GGA GGG CAG AAG GCT GGA GCA TTC ACC TGT CTC
AGC AAC AGT ATT TAC AGG ATC GAC TGC CAC TGG TCG GCT CCA
GAG CTG GGC CAG GAA TCC AGG GCC TGG CTC CTC TTT ACC AGT
AAC CAG GTG ACT GAA ATC AAA CAC AAA TGC ACC TTC TGG GAC
AGT ATG TGT ACC CTG GTG CTG CCT AAA GAG GAG GTG TTC TTA
CCT TTT GAC AAC TTC ACC ATC ACA CTT CAC CGC TGC ATC ATG
GGA CAG GAA CAG GTC AGC CTG GTG GAC TCA CAG TAC CTG CCC
AGG AGA CAC ATC AAG TTG GAC CCA CCC TCT GAT CTG CAG AGC
AAT GTC AGC TCT GGG CGT TGT GTC CTG ACC TGG GGT ATC AAT
CTT GCC CTG GAG CCA TTG ATC ACA TCC CTC AGC TAC GAG CTG
GCC TTC AAG AGG CAG GAA GAG GCC TGG GAG CAG GCC CGG CAC
AAG GAC CGT ATC GTT GGA GTG ACC TGG CTC ATC CTT GAA GCC
GTC GAA CTG AAT CCT GGT TCC ATC TAC GAG GCC AGG CTG CGT
GTC CAG ATG ACT TTG GAG AGT TAT GAG GAC AAG ACA GAG GGG
GAA TAT TAT AAG AGC CAT TGG AGT GAG TGG AGC CAG CCC GTG
TCC TTT CCT TCT CCC CAG AGG AGA CAG GGC CTC CTG GTC CCA
CGC TGG CAA TGG TCA GCC AGC ATC CTT GTA GTT GTG CCC ATC
TTT CTT CTG CTG ACT GGC TTT GTC CAC CTT CTG TTC AAG CTG
TCA CCC AGG CTG AAG AGA ATC TTT TAC CAG AAC ATT CCA TCT
CCC GAG GCG TTC TTC CAT CCT CTC TAC AGT GTG TAC CAT GGG
GAC TTC CAG AGT TGG ACA GGG GCC CGC AGA GCC GGA CCA CAA
GCA AGA CAG AAT GGT GTC AGT ACT TCA TCA GCA GGC TCA GAG
TCC AGC ATC TGG GAG GCC GTC GCC ACA CTC ACC TAT AGC CCG
GCA TGC CCT GTG CAG TTT GCC TGC CTG AAG TGG GAG GCC ACA
GCG TGA
GAAGGGACAG CCAGCCACTC AGTGCGTGGG CTTAGATTGG GAAGAGACCT
CCCAAGCAGC TTCCCCTCCT CCCCAGCCCC TGCCATTAC CCCTGCTGGC
CGTCCATCCC CAGGATCCAC TGTGGAGCCA AGCCCACAGA CCCGGCCTGA
TTCAGCTCTG ACACTCGCTG CGCTGCTCCG TTGTGAACTT TGGCCAAGTC
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GCCTAATCCC TAAGGAGACT GAGACAACT TGTCTGCAA TATCTATCCG
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CATGTCTCAA CCAACACTCT CCAAGCTGAA TTCTCAGGCT GAACTCACTG
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CAGGAGATGC TTGTGCCCTG AGGTATCTTT AAGAACTATC ACGGAATTGG
ACTGGGAGCT CAGGAGAGAG CTTGGTAGAC TGGCAGTGTC AGTGAAACAG
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AACCAACCTG GAACATGGGA GGTGTAAGT TCG

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SEQ ID NO: 3

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AGA GTG GCG GTG AAA CAG GTC TCC TGG TTC CTG ATC TAC AGC
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CAA GGA GGA GGA GGG CAG AAG GCT GGA GCA TTC ACC TGT CTC
AGC AAC AGT ATT TAC AGG ATC GAC TGC CAC TGG TCG GCT CCA
GAG CTG GGC CAG GAA TCC AGG GCC TGG CTC CTC TTT ACC AGT
AAC CAG GTG ACT GAA ATC AAA CAC AAA TGC ACC TTC TGG GAC
AGT ATG TGT ACC CTG GTG CTG CCT AAA GAG GAG GTG TTC TTA
CCT TTT GAC AAC TTC ACC ATC ACA CTT CAC CGC TGC ATC ATG
GGA CAG GAA CAG GTC AGC CTG GTG GAC TCA CAG TAC CTG CCC
AGG AGA CAC ATC AAG TTG GAC CCA CCC TCT GAT CTG CAG AGC
AAT GTC AGC TCT GGG CGT TGT GTC CTG ACC TGG GGT ATC AAT
CTT GCC CTG GAG CCA TTG ATC ACA TCC CTC AGC TAC GAG CTG
GCC TTC AAG AGG CAG GAA GAG GCC TGG GAG CAG GCC CGG CAC
AAG GAC CGT ATC GTT GGA GTG ACC TGG CTC ATC CTT GAA GCC
GTC GAA CTG AAT CCT GAA AAA AGA ATG TGA
CAAGCAGCCT GGTCTGTTCT TCCACCCCTA AAGGGCTGGC CTGGGCCCCAG
GGACACTGAT GAGACAACAT TGGTGAAGTG TCCCTTTTCA GTGCCTTTCC
CATTAAAGACC AGAAGGGACG CTTTTGACTG CAGGCTGTGG GTGGCTGGGT
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AGAGGTGTGG AGACATGGCA GCCAGCGGCA CACATGGCAG TGAGGACAGG
AAGCTGAGAG CTCACATCTC AACC AAAAGT TGAGTGAAC TGAAGTACTA
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ATGCCTCCCT AAACAGCTCG GCCAAATAGA GACCAAGTGT TCAAATAAAA
AAAA

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SEQ ID NO: 4

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CTCCAGGTTG GGGATGCCTC AGACTTGTG
ATG GGA CTG GGC AGA TGC ATC TGG GAA GGC TGG ACC TTG GAG
AGT GAG GCC CTG AGG CGA GAC ATG GGC ACC TGG CTC CTG GCC
TGC ATC TGC ATC TGC ACC TGT GTC TGC TTG GGA GTC TCT GTC
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AGC AAC CAG GCT CCT GGC GGC ACA CAT AAG TGC ATC TTG CCG
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TCT GGG AGG GAG CAG GTC AGC CTG GTG GAC CCG GAG TAC CTG
CCC CGG AGA CAC GTT AAG CTG GAC CCG CCC TCT GAC TTG CAG
AGC AAC ATC AGT TCT GGC CAC TGC ATC CTG ACC TGG AGC ATC
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CAC AGG GAT CAC ATT GTC GGG GTG ACC TGG CTT ATA CTT GAA
GCC TTT GAG CTG GAC CCT GGC TTT ATC CAT GAG GCC AGG CTG
CGT GTC CAG ATG GCC ACA CTG GAG GAT GAT GTG GTA GAG GAG
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TGC TTC CAG GCT CCC CAG AGA CAA GGC CCT CTG ATC CCA CCC
TGG GGG TGG CCA GGC AAC ACC CTT GTT GCT GTG TCC ATC TTT
CTC CTG CTG ACT GGC CCG ACC TAC CTC CTG TTC AAG CTG TCG
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TGT GAC CAT CAG GGC CTG GAG ACC CAG CAA GGA GTT GCC TGG
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TCC TGG ACA TTC TAG
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SEQ ID NO: 5

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CTC ACC AAC AAC ATT CTC AGG ATC GAT TGC CAC TGG TCT GCC
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CTG GCC TTC AAG AAG CAG GAA GAG GCC TGG GAG CAG GCC CAG
CAC AGG GAT CAC ATT GTC GGG GTG ACC TGG CTT ATA CTT GAA
GCC TTT GAG CTG GAC CCT GGC TTT ATC CAT GAG GCC AGG CTG
CGT GTC CAG ATG GCC ACA CTG GAG GAT GAT GTG GTA GAG GAG
GAG CGT TAT ACA GGC CAG TGG AGT GAG TGG AGC CAG CCT GTG
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GCATCCTGG

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SEQ ID NO: 6

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CTC ACC AAC AAC ATT CTC AGG ATC GAT TGC CAC TGG TCT GCC
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GGC AGT GAG TGC ACC GTC GTG CTG CCA CCT GAG GCA GTG CTC
GTG CCA TCT GAC AAT TTC ACC ATC ACT TTC CAC CAC TGC ATG
TCT GGG AGG GAG CAG GTC AGC CTG GTG GAC CCG GAG TAC CTG
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CCCTCTGCTC AGTGCCTGTG GGGAGCAGCC TCTACCCTCA GCATCCTGG

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We claim:

1. Isolated nucleic acid sequence which codes for or is complementary to a sequence which codes for interleukin-9 receptor.
2. The isolated nucleic acid sequence of claim 1, wherein said sequence codes for interleukin-9 receptor.
3. The isolated nucleic acid sequence of claim 2, wherein said sequence is cDNA.
4. The isolated nucleic acid sequence of claim 2, wherein said sequence codes for human interleukin-9 receptor.
5. The isolated nucleic acid sequence of claim 2, wherein said sequence codes for murine interleukin-9 receptor.
6. The isolated nucleic acid sequence of claim 2, selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.
7. The isolated nucleic acid sequence of claim 2, wherein said sequence is genomic DNA.
8. Vector comprising the isolated nucleic acid sequence of claim 2 operably linked to a promoter.
9. The vector of claim 8, further comprising a marker sequence.
10. The vector of claim 9, wherein said marker sequence is a resistance marker.
11. The vector of claim 8, wherein said vector is a plasmid.
12. Microorganism transfected with the nucleic acid sequence of claim 1.
13. The microorganism of claim 12, wherein said microorganism is Escherichia coli.
14. Cell line transfected with the nucleic acid sequence of claim 1.
15. The cell line of claim 14, wherein said cell line is a eukaryotic cell line.
16. The cell line of claim 15, wherein said eukaryotic

cell line is a CHO cell line.

17. The cell line of claim 15, wherein said eukaryotic cell line is a COS cell line.

18. The cell line of claim 15, wherein said eukaryotic cell line is a yeast cell line.

19. The cell line of claim 15, wherein said cell line is an insect cell line.

20. The cell line of claim 19, wherein said cell line is Spodoptera frugiperda.

21. The cell line of claim 19, wherein said nucleic acid sequence is incorporated into an expression vector.

22. The cell line of claim 21, wherein said expression vector is a baculovirus vector.

23. Process for producing an antibody which specifically binds to interleukin 9 receptor comprising immunizing a subject animal with the cell line of claim 15 under conditions favoring generation of antibodies which specifically bind to interleukin 9 receptor and isolating said antibodies from said animal.

24. Purified antibody produced by the process of claim 23.

25. Method for inhibiting effect of interleukin 9 on a subject comprising administering an amount of the antibody of claim 24 sufficient to inhibit binding of interleukin-9 to cells expressing interleukin 9 receptor, to a subject in need of inhibition of interleukin 9.

26. Method for determining a substance which binds to interleukin 9 receptor comprising contacting the cell line of claim 15 with a substance to be tested and determining binding or lack thereof to said cell line.

27. Method for determining an interleukin 9 receptor agonist comprising contacting the cell line of claim 15 with a substance to be listed and determining the affect thereon, wherein an affect characteristic of interleukin 9 is indicative of an interleukin 9 receptor agonist.

28. Method for determining an interleukin 9 antagonist comprising contacting the cell line of claim 15 with

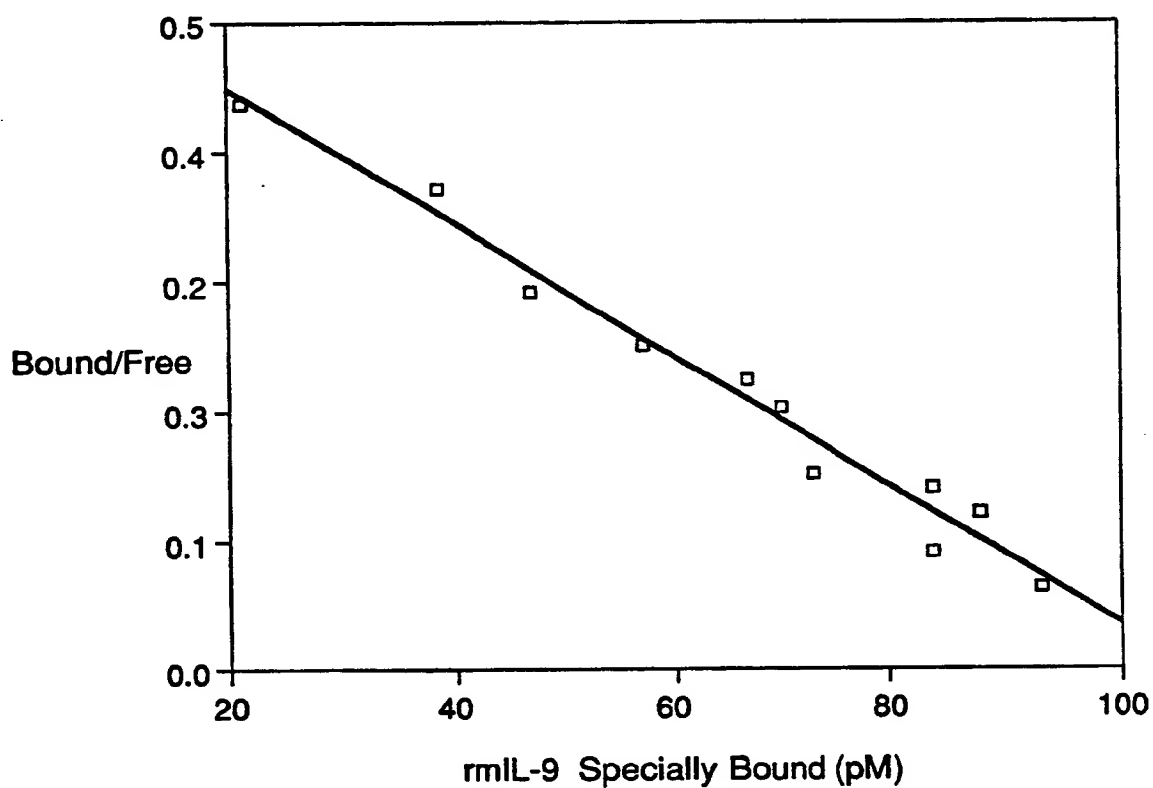
SUBSTITUTE SHEET

interleukin 9 and a substance to be tested and determining if said substance interferes with affect of interleukin-9 on said cell line, wherein said interference is indicative of an antagonist for interleukin-9.

29. Method for producing an antibody which specifically binds to interleukin 9 receptor comprising immunizing a non-human animal with an immunogenically effective form of interleukin 9 receptor in an amount sufficient to generate an antibody specific for interleukin 9 receptor, and purifying said antibody.

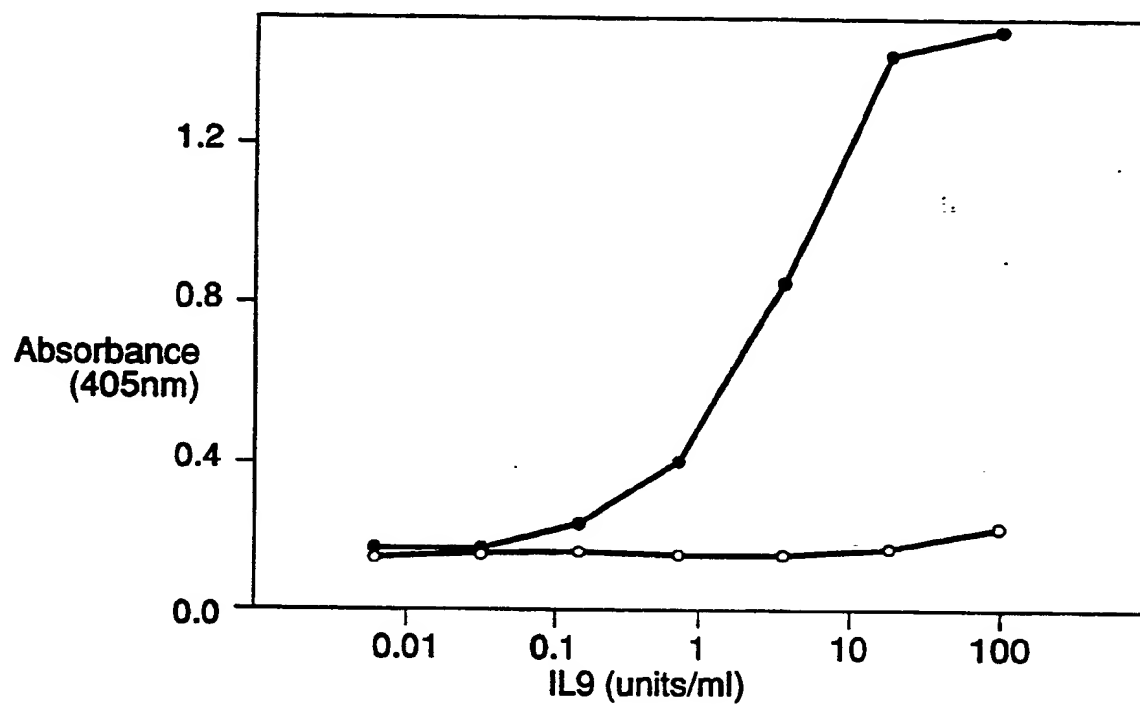
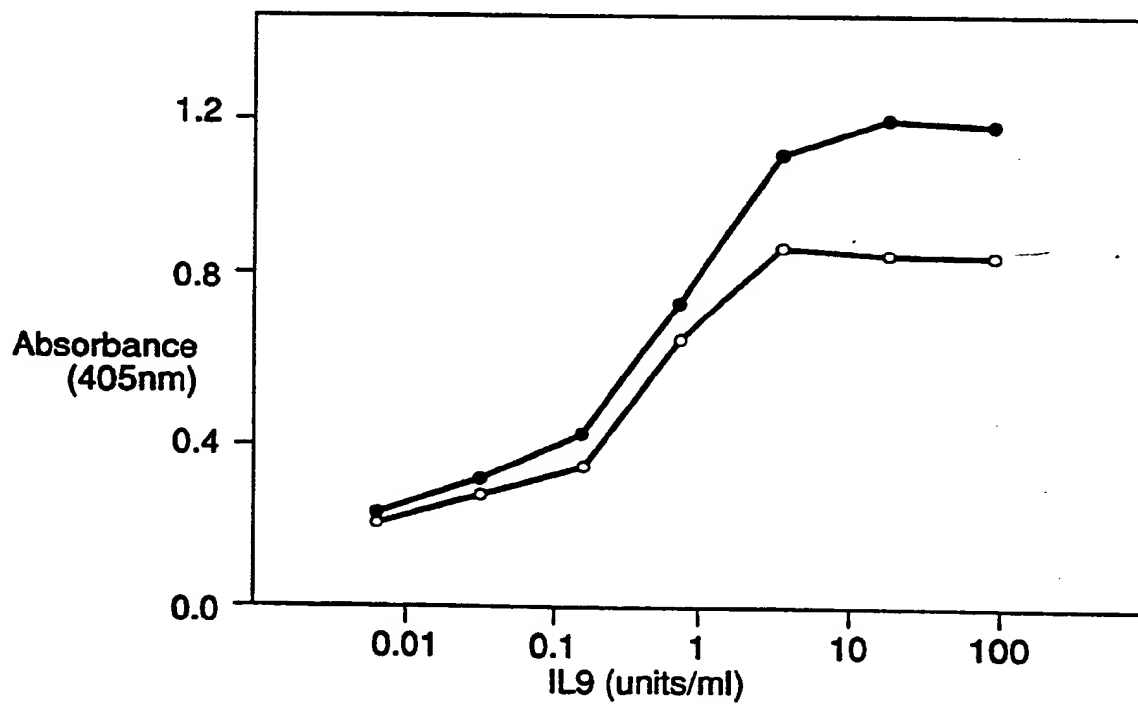
1/3

FIG. 1



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FIG. 3A**FIG. 3B**

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01720

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : 536/23.5; 435/7.2, 240.1, 320.1; 530/389.2; 424/85.8

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/7.2; 240.1, 320.1; 530/389.2; 424/85.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Sequence search of Sequence ID No's. 1-6; and antibodies to IL-9R

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	US, A, 5,116,951 (Druez et al), 26 May 1992, see all.	1-29
X	The Journal of Immunology, Vol. 145, Number 8, issued 15 October 1990, Druez et al, "Function and Biochemical Characterization of Mouse P40/IL-9 Receptors," pages 2494-2499, see all.	1-29
X	US, A, 4,675,285, (Clark et al), 23 June 1987, see all.	1-22
X	US, A, 5,030,576, (Dull et al), 09 July 1991, see all.	26-28



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

26 MAY 1993

Date of mailing of the international search report

08 JUN 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

GARNETTE D. DRAPER, PRIMARY EXAMINER

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01720

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Clin. Chem., Vol. 27, No. 11, issued 1981, Sevier et al, "Monoclonal Antibodies in Clinical Immunology," pages 1797-1806, see all.	23-25, 29
X	US, A, 4,707,443, (Nelson et al), 17 November 1987, see all.	23-25, 29
X	US, A, 4,636,463, (Altman et al), 13 January 1987, see all.	23-25,29
A,P	US, A, 5,132,109, (Dugas et al), 21 July 1992, see all.	1-29
A	Blood Reviews, Vol. 5, issued 1991, Kaczmariski et al, "The Cytokine Receptor Superfamily", pages 193-203, see all.	1-29

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/01720

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

(Telephone Practice)

Group I, claims 1-22 and 26-28, drawn to nucleic acids (NA), vectors, cell lines, and methods of receptor binding, and agonist/antagonist, classified in classes 536 and 435, subclasses 23.5, and 7.2, 240.1

Group II, claims 23-24 and 29, drawn to antibodies and a process of preparing such, classified in class 530, subclass 389.2

Group III, claim 25, drawn to a method of inhibiting IL-9 activity with anti-IL-9(R), classified in class 424, subclass 85.8

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/01720

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

C07H 15/12; C12N 5/00, 15/70; C07K 15/28; A61K 39/395; C12Q 1/00